



Early Journal Content on JSTOR, Free to Anyone in the World

This article is one of nearly 500,000 scholarly works digitized and made freely available to everyone in the world by JSTOR.

Known as the Early Journal Content, this set of works include research articles, news, letters, and other writings published in more than 200 of the oldest leading academic journals. The works date from the mid-seventeenth to the early twentieth centuries.

We encourage people to read and share the Early Journal Content openly and to tell others that this resource exists. People may post this content online or redistribute in any way for non-commercial purposes.

Read more about Early Journal Content at <http://about.jstor.org/participate-jstor/individuals/early-journal-content>.

JSTOR is a digital library of academic journals, books, and primary source objects. JSTOR helps people discover, use, and build upon a wide range of content through a powerful research and teaching platform, and preserves this content for future generations. JSTOR is part of ITHAKA, a not-for-profit organization that also includes Ithaka S+R and Portico. For more information about JSTOR, please contact support@jstor.org.

THE EFFECT OF SYMBIOSIS UPON SPORE FORMATION BY *BACILLUS WELCHII*, WITH SPECIAL REFER- ENCE TO THE PRESENCE OF THESE SPORES IN STOOLS *

J. P. SIMONDS

(From the Department of Bacteriology of the University of Texas, Galveston, and the Department of Pathology of Northwestern University Medical School, Chicago)

The surgical importance of the bacillus welchii has been recognized since Fraenkel¹ showed that it was the cause of gas phlegmon. Its significance for the internist became evident when it was shown by Tissier,² Passini,³ and, more conclusively, by Kendall and his coworkers⁴ that this organism may be the cause of severe diarrheas especially in infants, but also in adults.

The usual method of detecting the bacillus welchii in the stools is by means of the heated milk test, first used by Botkin,⁵ and perfected and employed very extensively by Kendall.⁶

The stools of infants suffering from gas bacillus diarrhea may be acid in reaction and yet contain large numbers of spores of this organism. The addition of sugar to the diet of such infants causes a serious and marked aggravation of the symptoms and an increase in the number of spores in the stools. Since it is well known that in artificial media the bacillus welchii does not sporulate except when such media are neutral or slightly alkaline and free from sugar, it seemed advisable to determine whether or not this bacillus could form spores in the presence of free acid or fermentable sugar in symbiosis with other organisms. Sittler⁷ has stated that the gas bacillus can produce spores in symbiosis with the colon bacillus under conditions and in media in which it would otherwise be unable to sporulate.

A number of different strains of the bacillus welchii, isolated from a variety of sources, were grown in broth and in sterilized suspensions of feces, with and without the addition of dextrose, in symbiosis with

* Received for publication September 22, 1914.

1. Ueber Gasphlegmone, Hamburg, 1893.
2. Ann. de l'Inst. Pasteur, 1905, 19, p. 273.
3. Ztschr. f. Hyg. u. Infektionskrankh., 1905, 49, p. 135.
4. Boston Med. and Surg. Jour., 1910, 163, p. 578; Ibid., 1912, 166, p. 75; Ibid., 1913, 169, p. 741.
5. Ztschr. f. Hyg. u. Infektionskrankh., 1894, 11, p. 421.
6. Ibid.
7. Centralbl. f. Bakteriologie Orig., 1908, 47, pp. 14, 145.

B. coli, *B. subtilis*, and *B. prodigiosus*. In no instance, did the symbiosis have any effect upon the spore-forming powers of any of the strains used.

The influence of the mixed fecal flora was next determined. The stools of a normal, healthy adult, which contained from 10-2,000 spores of the bacillus welchii per gram of dried feces, were available for systematic study. At frequent intervals, suspensions were made of these stools in sterile salt solution. The suspension was distributed in

TABLE 1
SHOWING THE INFLUENCE OF THE MIXED FECAL FLORA UPON SPORULATION BY THE *BACILLUS WELCHII* IN THE PRESENCE OF FERMENTABLE SUGARS

Fecal Suspension	Hours* Incuba- tion	Acidity* (Phenolph- thalein)	Dilutions†									
			1-100	1-200	1-400	1-800	1-1,600	1-3,200	1-6,400	1-12,800	1-25,600	1-51,200
Fresh.....			+	+	+	+	+	-	-	-	-	-
Plain.....	24	0.4	+	+	+	+	+	+	+	+	+	+
With 1 percent lactose..	24	3.0	+	+	+	+	+	+	+	+	+	-
Fresh.....			+	+	+	-	-	-	-	-	-	-
Plain.....	24	0.6	+	+	+	+	+	+	+	+	+	-
With 1 percent dextrose	24	5.4	+	+	+	-	-	-	-	-	-	-
With 1 percent lactose..	24	5.4	+	+	+	+	-	-	-	-	-	-
With 1 percent maltose.	24	4.4	+	+	+	-	-	-	-	-	-	-
Fresh.....			+	+	-	-	-	-	-	-	-	-
Plain.....	48	0.4	+	+	+	+	+	+	+	+	+	+
With 1 percent lactose..	48	2.6	+	+	+	+	+	+	+	+	+	+
With 1 percent dextrose	48	2.6	+	+	+	+	+	+	+	+	+	+
With 1 percent maltose.	48	2.6	+	+	+	+	+	+	+	+	+	+

* Expressed as the number of cubic centimeters of N/10 NaOH required to neutralize 100 c.c. of suspension.

† The sign + means that stormy fermentation was present, and that there was at least one spore in 1 c.c. of the dilution used.

50 c.c. amounts in large test tubes. In each lot, one tube of suspension was left without further treatment. To the others, there was added enough sterile solution of dextrose, lactose, or maltose, to make a 1 percent solution. The highest dilution of the suspension, which would produce stormy fermentation of milk when 1 c.c. was inoculated into a tube of milk and heated to 80 C. for fifteen minutes, was determined.

The tubes of suspension were then placed in a large desiccator or Novy jar in the bottom of which was a quantity of pyrogalllic acid wrapped in filter paper. Fifty to one hundred cubic centimeters of strong potassium hydroxid solution were then poured through a long

funnel into the jar which was then quickly closed. The air was strongly exhausted by means of a vacuum pump.

After twenty-four to forty-eight hours' incubation, the contents of each tube were thoroughly mixed and the highest dilutions, 1 c.c. of which would cause stormy fermentation of milk after heating to 80 C. for fifteen minutes, were determined for each tube. The occurrence of stormy fermentation in any tube of milk was considered proof of the presence of one or more spores of the *bacillus welchii* in 1 c.c. of the dilution of the suspension used.

The results of three characteristic experiments are shown in Table 1.

SUMMARY

In a plain suspension of feces containing the *bacillus welchii*, kept at 37 C. under anaerobic conditions for twenty-four to forty-eight hours, there is a marked increase in the number of spores of this organism.

In the presence of the mixed fecal flora, the *bacillus welchii* may sporulate even in the presence of free acid or fermentable carbohydrate.

The character of the associated flora is the most important factor determining the power of sporulation. When acid formers are so abundant that the acidity becomes such that 3 c.c. or more of N/10 NaOH are required to render 100 c.c. of medium neutral to phenolphthalein, there is no increase in the number of spores. When, on the other hand, acid-producing bacteria are absent or few in number so that less than 3 c.c. of N/10 NaOH will render 100 c.c. of suspension neutral, the *bacillus welchii* is able to produce spores in abundance.

That this ability to sporulate in the presence of free acid or fermentable sugar depends upon symbiotic relations of the *bacillus welchii* with one or more species, other than the colon bacillus, is indicated by the negative results obtained when a pure culture of the *bacillus welchii* was grown in symbiosis with a pure culture of the colon bacillus.